

IC20 Rec'd PCT/PTO SEP 26 2001

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

4296-146 US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/937414

INTERNATIONAL APPLICATION NO.
PCT/JP00/02034INTERNATIONAL FILING DATE
March 30, 2000 (30.03.00)PRIORITY DATE CLAIMED
March 31, 1999 (31.03.99)

TITLE OF INVENTION

DERMATOLOGICAL AGENT FOR EXTERNAL USE

APPLICANT(S) FOR DO/EO/US

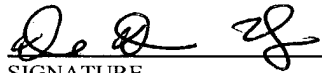
Murase, T. et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.137(a) or (b))		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/937414		PCT/JP00/02034		4296-146 US	
24. The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO				\$1000.00	
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO				\$860.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO				\$710.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)				\$690.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)				\$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	6 - 20 =	0	x \$18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$80.00	\$0.00	
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$990.00	
<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00	
SUBTOTAL =				\$990.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				+\$0.00	
TOTAL NATIONAL FEE =				\$990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL FEES ENCLOSED =				\$990.00	
				Amount to be refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$990.00 to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2165 A duplicate copy of this sheet is enclosed.					
d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
Diane Dunn McKay, Esq. Reg. No. 34,586 Mathews, Collins, Shepherd & Gould, P.A. 100 Thanet Circle, Suite 306 Princeton, NJ 08540 (609) 924-8555 Telephone (609) 924-3036 Facsimile			 SIGNATURE Diane Dunn McKay NAME 34,586 REGISTRATION NUMBER September 26, 2001 DATE		

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53) <div style="font-size: 24pt; font-weight: bold; text-align: center;">09/937414</div>		INTERNATIONAL APPLICATION NO. <div style="font-weight: bold; text-align: center;">PCT/JP00/02034</div>		<div style="text-align: right;"> 0016 Rec'd PCT/PTO SEP 26 2001 ATTORNEY'S DOCKET NUMBER 4296-146 US </div>	
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24. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :				CALCULATIONS PTO USE ONLY	
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO				\$1000.00	
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO				\$860.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO				\$710.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)				\$690.00	
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)				\$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	6 - 20 =	0	x \$18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$80.00	\$0.00	
Multiple Dependent Claims (check if applicable).				<input type="checkbox"/>	\$0.00
TOTAL OF ABOVE CALCULATIONS =				\$990.00	
<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00	
SUBTOTAL =				\$990.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				<input type="checkbox"/>	\$0.00
TOTAL FEES ENCLOSED =				\$990.00	
				Amount to be: refunded	\$
				charged	\$

a. ☒ A check in the amount of \$990.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2165 A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Diane Dunn McKay, Esq.
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 100 Thanet Circle, Suite 306
 Princeton, NJ 08540
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SIGNATURE
Diane Dunn McKay
 NAME
34,586
 REGISTRATION NUMBER
September 26, 2001
 DATE

09/19/2001 09:37:41
JC16 Rec'd PCT/PTO SEP 26 2001

Docket No.: 4296-146 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Murase, H. et al.

Serial No.: Herewith

Group Art Unit: TBD

Filed: September 26, 2001

Examiner: TBD

Title: DERMATOLOGICAL AGENT FOR EXTERNAL USE

Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination and prior to the calculation of the filing fee, please amend this application as follows:

In the claims:

Claim 3, line 2, please cancel "or claim 2".

Claim 4, line 2, please change "any of claims 1-3" to --claim 1--.

Claim 6, line 2, please change "any of claims 1-3" to --claim 1--.

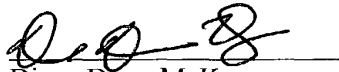
REMARKS

Claims 3, 4 and 6 have been amended to cancel multiple dependencies. Attached is a clean copy of claims 3, 4 and 6. Claims 1-6 are in this application.

Applicants believe that the claims would have been allowable as originally filed. Accordingly, applicants assert that no claims have been narrowed within the meaning of the Federal Circuit's recent decision in *Festo Corp. v. Shoketsu Kinzoku Kohyo Kabushiki Co.*, No. 95-1066, 2000 WL 1753646 (Fed. Cir. Nov. 29, 2000).

A prompt and favorable action on the merits is earnestly solicited. It is believed that no fee is required. The Commissioner is authorized to charge any deficiency or credit any overpayment to Deposit Account No. 13-2165.

Respectfully submitted,



Diane Dunn McKay
Reg. No. 34,586
Attorney for Applicant

DATE: September 26, 2001

MATHEWS, COLLINS, SHEPHERD & GOULD
100 Thanet Circle, Suite 306
Princeton, NJ 08540
(609) 924-8555 - Telephone
(609) 924-3036 - Facsimile

CLEAN COPY OF AMENDED CLAIMS

3. A dermatological agent for external use according to claim 1, which is an aqueous pharmaceutical preparation.

4. A dermatological agent for external use according to claim 1, which is an agent for preventing and curing dermatopathy.

6. A dermatological agent for external use according to claim 1, which is a cosmetic preparation.



5060

JC10 Rec'd PCT/PTO 27 DEC 2001

Docket No.: 4296-146 US

The undersigned certifies that this communication is being deposited with the United States Postal Service as prepaid first class mail in an envelope addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231 on October 26, 2001.


Diane Dunn McKay

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Murase, H. et al.

Serial No.: 09/937,414

Group Art Unit: TBD

Filed: September 26, 2001

Examiner: TBD

Title: DERMATOLOGICAL AGENT FOR EXTERNAL USE

Commissioner for Patents
Washington, DC 20231

SUPPLEMENTAL PRELIMINARY AMENDMENT

Sir:

Prior to examination and prior to the calculation of the filing fee, please amend this application as follows:

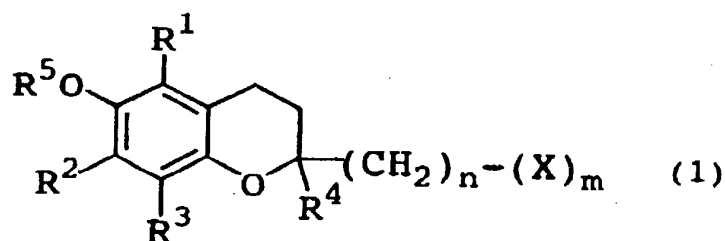
In the claims:

Please add claims 7-21.

7. (New) A method of preventing and treating a mammal which can comprise administering thereto an effective amount of the agent of claim 1.

8. (New) The method of claim 7 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

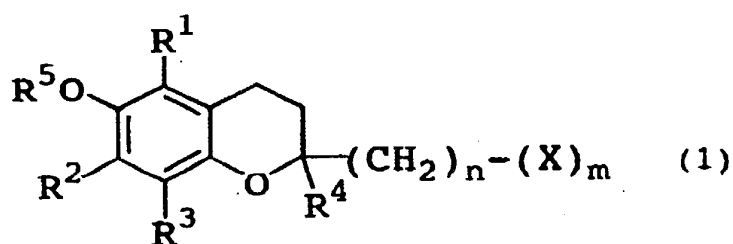
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(wherein R¹, R², R³, and R⁴, which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R⁵ represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

11. (New) The method of claim 10 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

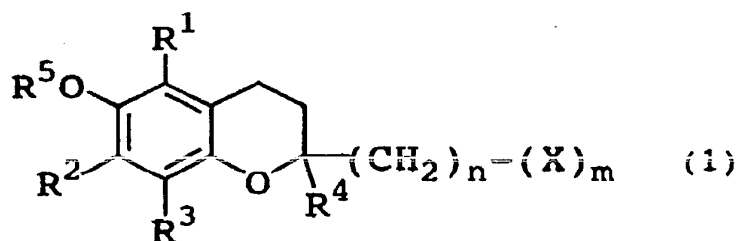
12. (New) A method for preventing and curing a disorder caused by ultraviolet light in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

13. (New) The method of claim 12 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

14. (New) A method for preventing and allowing the deposition of pigment in the skin in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)

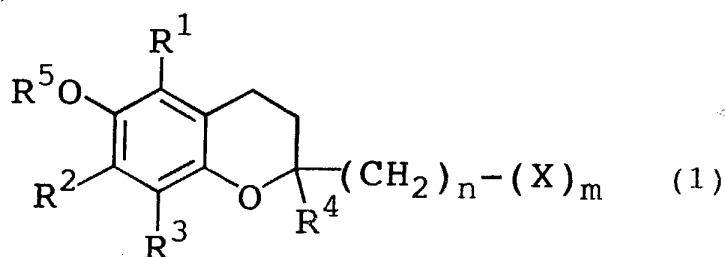


(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic

residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

15. (New) The method of claim 14 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

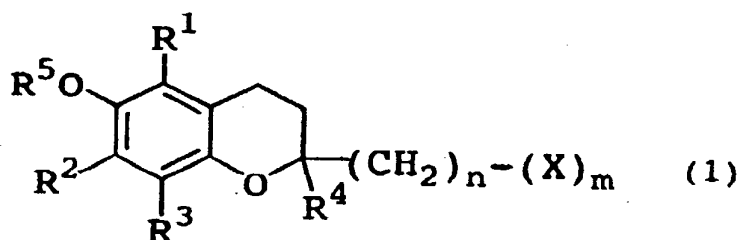
16. (New) A method for beautifying the skin in white in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

17. (New) The method of claim 16 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

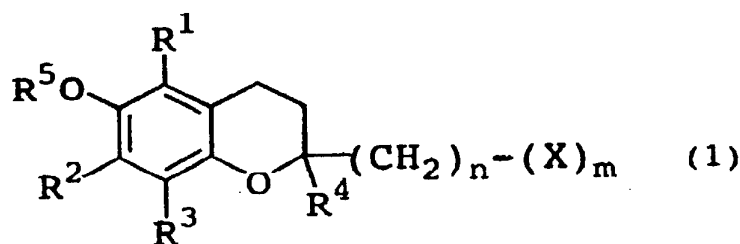
18. (New) A method for preventing the senescence of the skin in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

19. (New) The method of claim 18 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

20. (New) A method for activating cells in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



(wherein R¹, R², R³, and R⁴, which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R⁵ represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

21. (New) The method of claim 20 wherein said chromanol glycoside is 2-(α-D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β-D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α-D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.


REMARKS

New claims 7-21 have been added. Attached is a clean copy of new claims 7-21. Claims 1-21 are in this application. No new matter has been entered.

Applicants believe that the claims would have been allowable as originally filed. Accordingly, applicants assert that no claims have been narrowed within the meaning of the Federal Circuit's recent decision in *Festo Corp. v. Shoketsu Kinzoku Kohyo Kabushiki Co.*, No. 95-1066, 2000 WL 1753646 (Fed. Cir. Nov. 29, 2000).

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Respectfully submitted,


 Diane Dunn McKay
 Reg. No. 34,586
 Attorney for Applicant

DATE: October 26, 2001

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Princeton, NJ 08540
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(609) 924-3036 - Facsimile

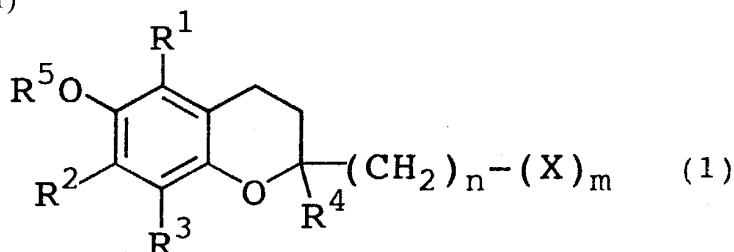
060357414 0131303

7. A method of preventing and treating a mammal which can comprise administering thereto an effective amount of the agent of claim 1.

8. The method of claim 7 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

9 The method of claim 8 wherein said agent is an aqueous pharmaceutical preparation.

10. A method for preventing and curing dermatopathy in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



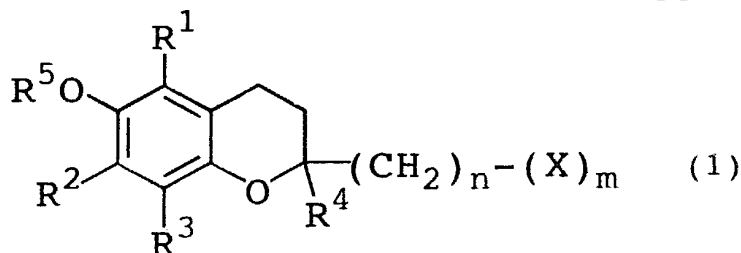
(wherein R¹, R², R³, and R⁴, which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R⁵ represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

11. The method of claim 10 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

15. The method of claim 14 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

16. A method for beautifying the skin in white in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)

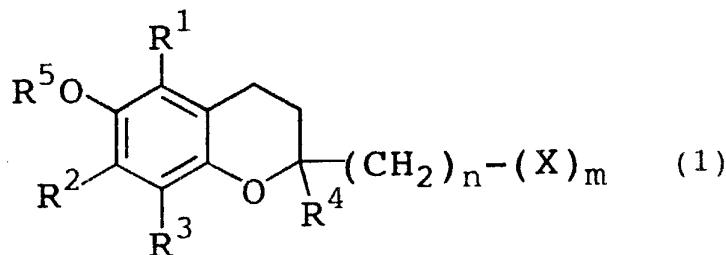


(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, x represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0-6, and m represents an integer in the range of 1-6).

17. The method of claim 16 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

18. A method for preventing the senescence of the skin in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)

20. A method for activating cells in a mammal which comprises administering thereto an effective amount of a dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



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21. The method of claim 20 wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

1/pts

DESCRIPTION

DERMATOLOGICAL AGENT FOR EXTERNAL USE

Technical Field

5 This invention relates to a novel dermatological agent for external use. More particularly, this invention relates to a dermatological agent for external use having a water-soluble chromanol glycoside as an effective component thereof.

Background Art

10 The skin is susceptible of various forms of stress such as ultraviolet light, heat, and chemical substances which exist in the environment because it is situated in the outermost surface of the human body. Among other forms of stress mentioned above, the ultraviolet light (particularly the UVB
15 having a wavelength region of 290 - 320 nm) is reputed to generate active oxygen and free radicals on the skin surface and in the cutaneous tissues and form the cause for sunburn and cutaneous cancer ("Active Oxygen and Morbidity" compiled and written by Masayasu Inoue and published by Gakkai Shuppan
20 Center on October 1, 1992, pp. 567 - 576). Particularly, since the amount of the ultraviolet light that reaches the surface of the earth in consequence of the fracture in the ozonosphere has been continuing to increase in recent years, the protection of the skin with the ultraviolet absorber is no longer
25 satisfactory and the necessity for eliminating the active oxygen and free radicals which have been generated as within the cutaneous tissues has been gaining in importance. Further, it has recently come to light that the cytokine, an inflammatory chemical mediator, is derived by an ultraviolet light and
30 that this mediator incites derivation of such immunocytes as leukocytes and consequently gives rise to a local inflammatory reaction and exerts heavy damage on the skin

fibroblasts which synthesize such a matrix component as collagen in the skin. As the substances that activate cutaneous cells and prevent them against senescence, vitamin C, vitamin E, retinoic acid, and retinol derivatives have
5 been known. These substances invariably have a dubious quality in stability, percutaneous absorbency, and teratogenicity and, as such, find utility in an extremely limited range.

The chromanol glycoside which is used in this invention
10 is a known compound (JP-A-07-118, 287, JP-A-09-249,688, and JP-A-11-21,291). The chromanol glycoside is obtained by substituting an alcohol for the phytyl group at the 2 position of the chroman ring of α -tocopherol which is a typical vitamin E and further linking a saccharum to the alcohol. It possesses
15 high solubility in water and excellent resistance to oxidation. It has never been known, however, to utilize the chromanol glycoside mentioned above for the prevention of such cutaneous disorders as described above and as dermatological agent for external uses as curing agents and cosmetic articles.

20 This invention, initiated in view of the problematic points incurred by the prior art as described above, has as an object thereof the provision of a novel dermatological agent for external use which is capable of effectively acting to repress and cure the dermopathy caused as by the ultraviolet
25 light, for example, at a small application rate without entailing any side effect.

Another object of this invention is to provide a novel dermatological agent for external use which is capable of effectively eliminating the active oxygen and free radicals
30 forming the cause for the local cutaneous inflammation due to the ultraviolet light and, at the same time, repressing the production of a cytokine to be derived therefrom.

(wherein R¹, R², R³, and R⁴, which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R⁵ represents a hydrogen atom, a lower alkyl group, or a lower acyl group, X represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0 - 6, and m represents an integer in the range of 1 - 6).

10 This invention also concerns the dermatological agent
for external use mentioned above, wherein the chromanol
glycoside mentioned above is 2-(α -D-glucopyranocyl)methyl
-2,5,7,8-tetramethyl chroman-6-ol,
2-(β -D-galactopyranocyl)methyl-2,5,7,8-tetramethyl
15 chroman-6-ol, 2-(β -D-fructofuranosyl)methyl-2,5,7,8-
-tetramethyl chroman-6-ol, or 2-(α -D-mannopyranosyl)methyl
-2,5,7,8-tetramethyl chroman-6-ol.

This invention further concerns the dermatological agent for external use mentioned above, which is an aqueous preparation.

This invention further concerns the dermatological agent for external use mentioned above, which is an agent for preventing and curing dermopathy.

25 This invention further concerns the dermatological agent
for external use mentioned above, which is an agent for
preventing and curing disorders caused by the ultraviolet
light, an agent for preventing and improving deposition of
pigment in the skin, an agent for beautifying the skin in
white, an agent for preventing the skin from senescence, and
30 an agent for activating the cells.

This invention further concerns the dermatological agent for external use, which is a cosmetic article.

represented by X, such saccharic residues as maltose, lactose, cellobiose, raffinose, xylobiose, and sucrose which have linked thereto two to four monosaccharides may be cited. Among other saccharic residues mentioned above, such monosaccharic residues as glucose, galactose, fucose, xylose, rhamnose, mannose, and fructose prove particularly favorable. The hydrogen atom of the hydroxyl group in the saccharic residue represented by X may be substituted with a lower alkyl group, preferably a lower alkyl group of 1 - 8 carbon atoms, or a lower acyl group, preferably a lower acyl group of 1 - 10 carbon atoms. Then, n represents an integer in the range of 0 - 6, preferably 1 - 4 and m represents an integer in the range of 1 - 6, preferably 1 - 3. As preferred concrete examples of the chromanol glycosides represented by the general formula (1), 2-(α -D-glucopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -L-fucopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(α -L-rhamnopyranosyl)-methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-xylopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-glucopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-fructo-furanosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol may be cited.

25 The chromanol glycoside to be used in this invention is produced by the enzyme reaction which comprises causing a 2-substituted alcohol represented by the following general formula (2);

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25

ATCC 9995 may be cited.

(3) In the case of linking a glucose residue to a 2-substituted alcohol with a β -bond:

(a) The oligosaccharide formed of such a β -bond
5 as cellobiose, curdlan, or laminaran is preferred to be acted
on by β -glucosidase, EC 3.2.1.21.

(b) The cellobiose in the presence of phosphoric acid is preferred to be acted on by cellobiose phosphorylase, EC 2.4.1.20.

10 (4) In the case of linking galactose residue to a
2-substituted alcohol with an α -bond:

The melibiose or the raffinose is preferred to be acted on by α -galactosidase, EC 3.2.1.22.

(5) In the case of linking a galactose residue to a 2-substituted alcohol with a β -bond:

(a) The lactose or the like is preferred to be acted on by β -galactosidase, EC 3.2.1.23.

(b) The arabinogalactan or the like is preferred to be acted on by endo-1,4- β -galactanase, EC 3.2.1.89.

20 (6) In the case of linking a fructose residue to a
2-substituted alcohol with a β -bond:

(a) The sucrose, raffinose, melibiose, or the like is preferred to be acted on by levansucrase, EC 2.4.1.10.

(b) The sucrose is preferred to be acted on by
25 β -fructofuranosidase, EC 3.2.1.26.

(c) The inulin or the like is preferred to be acted on by inulin fructotransferase, EC 2.4.1.93.

The reaction conditions in the reaction mentioned above are variable with the kinds of chromanol glycoside and enzyme to be used therein. When a chromanol glycoside answering the general formula (1) with 1 for m is synthesized by using α -glucosidase, for example, the 2-substitute alcohol is

The 2-substituted alcohol in the reaction solution is preferred to have a saturated concentration or a concentration approximating closely thereto. The kind of sugar to be favorably used is a low molecular sugar on the order of maltose through maltotetraose, preferably maltose. The concentration of the sugar is in the range of 1 - 70 (mass/volume)%, preferably 30 - 60 (mass/volume)%. The pH value is in the range of 4.5 - 7.5, preferably of 5.0 - 6.5. The reaction temperature is in the range of 10 - 70°C, preferably 30 - 60°C. Of course, these conditions are affected by the amount of the enzyme to be used, for example. After the reaction is completed, the chromanol glycoside aimed at by the reaction is obtained in high purity by subjecting the reaction solution to column chromatography using a substance (made by Japan Organo Co., Ltd. and sold under the trademark designation of "XAD") as a carrier.

When a chromanol glycoside answering the general formula (1) with 1 for m is synthesized by the use of cyclodextrin glucanotransferase, for example, the relevant reaction

— 12 —

the reaction time is in the range of 0.1 - 40 hours, preferably in the range of 1 - 24 hours. Only, these conditions are affected by the amount of an enzyme to be used. Then, by subjecting the liquid resulting from the aforementioned treatment with glucoamylase to column chromatography using a substance (made by Japan Organo Co., Ltd. and sold under the trademark designation of "XAD") as a carrier, it is made possible to obtain a chromanol glycoside answering the general formula (1) with 1 for m in high purity.

In obtaining a chromanol glycoside answering the general formula (1) with 2 for m, by causing β -amylase (EC 3.2.1.2) to act on chromanol glycosides having the form of a mixture answering the general formula (1) with integers 1 to 8 for m which are obtained with cyclo-dextrin glucanotransferase under the same conditions as mentioned above, it is made possible to obtain exclusively a chromanol glycoside answering the general formula (1) with 1 or 2 for m. At this time, the reaction temperature is in the range of 20 - 70°C, preferably in the range of 30 - 60°C and the reaction time is in the range of 0.1 - 40 hours, preferably in the range of 1 - 24 hours. Only, these conditions are affected by the amount of an enzyme to be used. By subjecting the liquid resulting from the treatment with β -amylase to column chromatography using a substance (made by Japan Organo Co., Ltd. and sold under the trademark designation of "XAD") as a carrier, it is made possible to obtain a chromanol glycoside answering the general formula (1) with 2 for m in high purity and to obtain a chromanol glycoside answering the general formula (1) with 1 for m as well.

In obtaining chromanol glycosides answering the general formula (1) with integers of not less than 3 for m, by subjecting chromanol glycosides obtained with cyclodextrin

glucano-transferase and having the form of a mixture answering the general formula (1) with integers 1 to 8 for m to fractionation chromatography using HPLC, for example, it is made possible to obtain chromanol glycosides of high purity
5 respectively for the integers of m.

The modes of embodiment described above have depicted the cases of linking a glucose residue or a maltooligosaccharide residue as a sugar residue to the 2-substituted alcohol. In the case of linking a galactose residue, mannose residue, or fructose residue as a sugar residue to the 2-substituted alcohol, by following the procedure of the mode of embodiment described above while using a proper enzyme mentioned already in the paragraph dealing with enzymes capable of catalyzing the sugar transferring action, it is made possible to obtain the target chromanol glycoside in high purity (JP-A-09-249,688 and JP-A-11-21,291).

The chromanol glycoside to be used in this invention may be also produced by subjecting the aforementioned 20 2-substituted alcohol having the hydroxyl group at the 6 position thereof protected with a protection group (hereinafter referred to as "sugar acceptor") and a sugar derivative having a leaving group introduced to the anomer position thereof and another hydroxyl group thereof protected 25 with a protection group (hereinafter referred to as "sugar donor") to a condensation reaction in accordance with the method disclosed in JP-B-10-75,599 (method of organic synthesis).

As concrete examples of the protection group serving
30 the purpose of protecting the hydroxyl group at the 6 position
of the sugar acceptor to be used in the reaction mentioned
above, acetyl group, benzoyl group, pivaloyl group,

chloroacetyl group, levulinoyl group, benzyl group, p-methoxybenzyl group, allyl group, t-butyldimethylsilyl group, t-butyldiphenylsilyl group, trimethylsilyl group, and trityl group may be cited. Among other protection groups
5 mentioned above, acetyl group and benzoyl group prove particularly advantageous.

As concrete examples of leaving group to be introduced to the anomer position of the sugar donor for use in the reaction mentioned above, halogen atoms such as chlorine, bromine,
10 and fluorine, sulfur compounds such as thiomethyl group, thioethyl group, and thiophenyl group, and trichloroacetoimide group may be cited. Among other leaving groups mentioned above, bromine, chlorine, thiomethyl group, thioethyl group, thiophenyl group, and trichloroacetoimide
15 group prove particularly advantageous. As concrete examples of the protection group serving the purpose of protecting the hydroxyl group at a position other than the anomer position, acyl type protection groups such as acetyl group, benzoyl group, pivaloyl group, chloroacetyl group, and levulinoyl
20 group and ether type protection groups such as benzyl group, p-methoxybenzyl group, allyl group, t-butyldimethylsilyl group, t-butyldiphenylsilyl group, trimethylsilyl group, and trityl group may be cited. Among other protection groups mentioned above, acyl type protection groups, particularly
25 acetyl group, prove particularly favorable.

These sugar donors can be easily prepared by introducing a protection group into any of the relevant hydroxyl groups by the universally known method and then substituting the atom or group at the anomer position with a leaving group.

30 To illustrate the condensation reaction between the sugar acceptor and the sugar donor mentioned above, the reaction is started with the action of solving the sugar

acceptor and the sugar donor in a non-polar solvent. The amounts of the sugar acceptor and the sugar donor necessary for charging the relevant reaction vessel are properly to be such that the molar ratio of the sugar acceptor to the sugar donor falls in the range of 1.0 - 1.5, preferably in the range of 1.1 - 1.3. As concrete examples of the non-polar solvent, methylene chloride and benzene may be cited.

Then, the sugar donor and the sugar acceptor are subjected to a condensation reaction under the anhydrous condition in the presence of an activating agent. As concrete examples of this activating agent, trifluoroboric acid-ether complex, silver perchlorate, silver trifluoromethane sulfonate, mercury bromide, mercury cyanide, N-iodosuccinic acid imide-trifluoromethane sulfonic acid, dimehylmethylthiosulfonium trifurate, and p-toluene sulfonic acid may be cited. When bromine is adopted as the leaving group of the sugar derivative in particular, it is advantageous to use such a heavy metal salt as silver perchlorate. The reaction temperature is in the range of 5 - 30°C, preferably in the range of 10 - 25°C and the reaction time is in the range of 12 - 48 hours, preferably in the range of 20 - 30 hours.

Then, by purifying the resultant reaction product as by silica gel column chromatography and depriving the purified reaction product of the protection group as with sodium hydroxide and methanolic hydrochloric acid, it is made possible to obtain 2-(β -L-fucopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(α -L-rhamnopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-xylopyranosyl)methyl-2,5,7,8-tetramethyl

The chromanol glycoside which is obtained by the enzyme method or the method of organic synthesis mentioned above is generally an amphiphilic molecule which possesses extremely high water solubility (about 100 g/100 ml) and 5 abunds in oil solubility. In other words, the chromanol glycoside according to this invention may well be called a water soluble vitamin E endowed with high affinity for lipids. Thus, the chromanol glycoside according to this invention, 10 unlike the conventional vitamin E derivatives which are insoluble or sparingly soluble in water, retains the high affinity for lipids even when used as dissolved in water and, therefore, exhibits veritably excellent percutaneous absorbency, permeates cell membranes, and further infiltrates 15 the cells. As a result, it prevents the dermatopathy or rapidly ameliorates the morbidity by reinforcing the antioxidation preventive system in the living body, effectively eliminating the active oxygen and free radicals generated on the surface and in the tissue of the skin by the ultraviolet light, and 20 effectively repressing the production of the cytokine possibly induced in the site of local inflammation as well. Since the chromanol glycoside is capable of activating very effectively the fibroblast that synthesizes such a matrix component as the collagen in the skin, it serves the purpose 25 of activating the metabolism of collagen and hyaluronic acid, enhancing the flexibility and elasticity of the cutaneous cells, and accelerating the turnover, with the result that the sedimentation of pigment in the skin will be repressed and the beautification of the skin in white will be promoted. 30 Further, the chromanol glycoside which is obtained by the reaction mentioned above is prominently improved also in thermal stability, pH stability, and stability of

preservation as compared with tocopherol, Trolox®, and 2-substituted alcohol.

The external agent of this invention can be utilized in the form of a pharmaceutical preparation or a cosmetic preparation.

For application to pharmaceutical preparations, the dermatological agent for external use of this invention can be used as agents for preventing and curing cutaneous inflammation, sunburn, early senescence, cutaneous cancer, and keratosis caused by the stress of ultraviolet light, heat, and chemical substance, an agent for preventing and allaying sedimentation of pigment in the skin, an agent for beautification of the skin in white, an agent for preventing and allaying the formation of wrinkles and sags, an agent for preventing cutaneous senescence, and an agent for preventing and curing the dermopathy such as an agent for activating cutaneous cells. In this case, this dermatological agent for external use can be percutaneously administered to target sites or cites in the proximity thereof in the form of a liquid preparation such as lotion, suspension, or emulsion, in the form of a semi-solid preparation such as gel, cream, or ointment, or in the form of a solid preparation such as powder, dust, or granule transformed to a solution prior to use by application to a surface. The form of preparation and the mode of administration mentioned above may be properly selected by physicians in charge of relevant treatments so as to suit the age, sexuality, constitution, symptom, and time of treatment of each of the patients.

For application to cosmetic preparations, the dermatological agent for external use of this invention can be manufactured into cosmetic articles of such semi-solid to solid state as liquid, paste, gel, and cream and can be

utilized specifically as cosmetic liquid, lotion, emulsion, cream, pack, cleanser, foundation, lip stick, shampoo, rinse, and treatment.

The dermatological agent for external use of this invention can be produced in accordance with the customary procedure which comprises properly combining the chromanol glycoside mentioned above with pharmaceutical components or cosmetic components in popular use. Specifically, pharmaceutical or cosmetic preparations in such liquid forms as aqueous solution, non-aqueous solution, suspension, ribosome, and emulsion and in such semi-solid or solid forms as paste, gel, and cream can be produced by suitably combining the chromanol glycoside mentioned above with buffers such as purified water and phosphate buffer, physiological salt solutions such as physiological saline solution, Ringer's solution, and Locke's solution, animal and plant oils such as lanolin, mink oil, horse oil, almond oil, castor oil, jojoba oil, meadow-foam oil, olive oil, sesame oil, and coconut butter, mineral oil, synthetic oils such as polyoxyethylene polyoxypropylene glycol, isopropyl myristate, isopropyl palmitate, cetostearyl isooctanate, and alkyl esters of isostearic acid, sterols such as cholesterol, lanolin alcohol, and phytosterol and derivatives thereof, waxes such as solid paraffin, ceresin, spermatoc wax, bees wax, and carnauba wax, hydrocarbon oils such as liquid paraffin and squalane, higher fatty acids such as lauric acid, stearic acid, and oleic acid, lower alcohols such as ethanol, higher alcohols lauryl alcohol, cetanol, cetostearyl alcohol, and oleyl alcohol, polyhydric alcohols such as glycerin, sorbit, propylene glycol, and 1,3-butylene glycol, surfactants such as sulfates of polyoxyethylene alkyl ethers, 2-alkyl-N-carboxymethyl-N-hydroxyethyl imidazolinium

betaine, acyl-L-glutamates of -coconut oil fatty acid, higher alcohol ethers of polyoxyethylene, and castor oil cured with polyoxyethylene, moisture retaining agents such as hyaluronates, pyrrolidone carboxylates, and hydrolyzed collagen solution, bodying agents such as seaweed extract, carageenan, xanthan gum, polyvinyl alcohol, and carboxyl vinyl polymers, antiseptic-bactericidal agents such as alkyl esters of oxybenzoic acid, cetylpyridinium chloride, benzalkonium chloride, alkyltrimethyl ammonium chlorides, phenoxy ethanol, triclosan, trichlorocarbanilidem, and zinc pyrithion, anti-oxidizing agents such as BHT, BHA, vitamins of A series, C series, E series, and derivatives, ultraviolet absorbents such as benzophenone derivatives, paraaminobenzoic acid derivatives, methoxy cinnamic acid derivatives, and urocanic acid, cation rinsing agents such as cationized dextran, animal plant extracts such as placenta extract, cock's comb extract, arnica extract, aloe extract, seaweed extract, camomile extract, licorice extract, cinchona extract, garlic extract, and melissa extract, and pigments and perfumes such as talc, kaolin, mica, bentonite, titanium mica, titanium oxide, iron oxide red, and iron oxide, and subjecting the resultant mixtures to solution, dispersion, emulsion, and intermixing.

Though the concentration of the chromanol glycoside contained in the dermatological agent for external use of this invention is variable with the mode of administration, the kind and seriousness of a disease, and the dosage aimed at, it is generally in the range of 0.1 - 90 mass %, preferably in the range of 1 - 80 mass %, based on the total mass of the raw materials involved. If the concentration of the chromanol glycoside exceeds the upper limit of the range mentioned above, the excess will be at a disadvantage in failing

5 The dosage of the dermatological agent for external use
of this invention varies with the age, body weight, and symptom
of a patient, the mode of administration aimed at, the effect
of cure, and the duration of treatment and ought to be
accurately determined by a physician. The dosage as reduced
10 to the content of chromanol glycoside is generally in the
range of 0.01 - 1000 mg/kg of body weight/day. This dosage
is given to a patient wholly once or several times as split
into as many portions daily.

As the chromanol glycoside, the following compounds were used. These compounds were produced by the methods described in the specific pieces of literature indicated in the parentheses following the respective names of the compounds

2-(α -D-Glucopyranosyl)methyl-2,5,7,8-tetramethyl
chroman-6-ol (JP-A-07-118,287)

2-(β-D-Galactopyranosyl)methyl-2,5,7,8-tetramethyl
chroman-6-ol (JP-A-09-249,688)

2-(β -D-Fructofuranosyl)methyl-2,5,7,8-tetramethyl
30 chroman-6-ol (JP-A-11-21,291)

2-(α -D-Mannopyranosyl)methyl-2,5,7,8-tetramethyl

culture medium added in a unit volume of 100 μ l to the wells, the contents of the wells were cultured for 72 hours. After the elapse of 72 hours from thence, the neutral red reagent (0.015%) was added in a unit volume of 100 μ l to the wells and the resultant contents of the wells were cultured for 3 hours. After the elapse of 3 hours from thence, the culture medium was removed and a fixing solution (aqueous solution containing 0.5% of formaldehyde and 0.1% of calcium chloride) was added in a unit volume of 200 μ l to the wells. The resultant contents of the wells were fixed for one minute and the fixing solution was removed from the wells. Then, an extracting solution (aqueous solution containing 50% of ethanol and 1% of acetic acid) were added in a unit volume of 100 μ l to the wells. The resultant contents of the wells were left standing for 20 minutes and assayed with a micro-plate reader for the absorbency at 490 nm and visually examined to count the number of live cells. On the basis of the results of the assay, the relative survival ratios of the samples were determined, with the number of cells in the groups of wells not irradiated with the ultraviolet light taken as 100%. The results are shown in Table 1.

Table 1

		Survival ratio (%)	
		V79	NB1RGB
Control group		21 \pm 6	45 \pm 13
Chromanol glycoside -added group	TMG	75 \pm 8*	78 \pm 16*
	TMGA	83 \pm 7*	83 \pm 14*
	TMFR	89 \pm 9*	81 \pm 12*
	TMMA	96 \pm 9*	79 \pm 8.7*

*: P < 0.05 (As compared with control group)

Test for confirming effect of repressing ultraviolet light (UVB)-induced cytokine

1. Method for testing effect of preventing ultraviolet light (UVB)-induced cytokine

5 Cornified cells of normal human neonate foreskin scurfskin (freeze-preserved product, made by Kurabo K.K.) were adjusted to a cell density of 1.0×10^5 pieces/ml in a HuMedia-KG2 culture medium (made by Kurabo K.K.), sown in a unit volume of 2 ml in the component wells of a 6-hole plate, and cultured in an atmosphere of 5% CO₂ at 37°C for 24 hours. After completion of the culture, the culture medium was removed and the wells were each washed twice with 2 ml of Hanks buffer. Some of these wells to which the Hanks buffer alone was added in a unit amount of 1 ml formed a control group and the remainders of them to which the Hanks buffer containing 0.1 mM of a given sample was added in a unit amount of 1 ml formed a sample-added group. The groups each consisted of 8 samples. They were irradiated with a UVB (312 nm) emitted from an ultraviolet lamp (made by Cosmo Bio K.K.) at a dosage of 30 mJ/cm². The amount of the energy so emitted was measured by the use of an ultraviolet light intensity meter (made by Topcon K.K. and sold under the trademark designation of "UVR-2").

20 Immediately after completion of the irradiation, the wells were each washed twice with 2 ml of Hanks buffer. With a HuMedia-KG2 culture medium added in a unit volume of 1 ml to the wells, the contents of the wells were cultured in an atmosphere of 5% CO₂ at 37°C for 6 hours.

2. Method for testing effect of curing ultraviolet light (UVB) disorder

30 Cornified cells of normal human neonate foreskin scurfskin (freeze-preserved product, made by Kurabo K.K.) were adjusted to a cell density of 1.0×10^5 pieces/ml in a

HuMedia-KG2 culture medium (made by Kurabo K.K.), sown in a unit volume of 2 ml in the component wells of a 6-hole plate, and cultured in an atmosphere of 5% CO₂ at 37°C for 24 hours. After completion of the culture, the culture medium was removed and the wells were each washed twice with 2 ml of Hanks buffer. The Hanks buffer alone was added in a unit volume of 1 ml to the wells. They were irradiated with a UVB (312 nm) emitted from an ultraviolet lamp (made by Cosmo Bio K.K.) at a dosage of 30 mJ/cm². The amount of the energy so emitted was measured by the use of an ultraviolet light intensity meter (made by Topcon K.K. and sold under the trademark designation of "UVR-2"). . Some of these wells to which the Hanks buffer alone was added in a unit amount of 1 ml formed a control group and the remainders of them to which the Hanks buffer containing 0.1 mM of a given sample was added in a unit amount of 1 ml formed a sample-added group. The groups each consisted of 8 samples. The contents of the wells were cultured in an atmosphere of 5% CO₂ at 37°C for 6 hours.

3. Determination of interleukin-1 α (IL-1 α)

20 The supernatant occurring at the end of 6 hours' culture
was recovered and centrifuged at 1000 rpm for 5 minutes. The
concentration of the IL-1 α in the resultant supernatant was
determined by the use of an ELISA kit made by ENDOGENE Corp.
The significance was verified by the t-test and was applied
25 to the groups yet to be treated. Table 2 shows the results
of the test for the effect of repressing the production of
IL-1 α .

Table 2

	Amount of IL-1 α produced (μ g/ml)	
	Effect of prevention	Effect of curing
Control group	29.9 \pm 6.2	28.9 \pm 4.5
TMG	14.5 \pm 3.5*	18.5 \pm 4.2*
Ascorbic acid	16.5 \pm 2.8*	31.7 \pm 5.0
Glutathione	7.2 \pm 3.4**	36.5 \pm 1.6

Means \pm S.E.

*: $p < 0.05$, **: $p < 0.01$ (invariably as compared with
5 the control group)

Though the chromanol glycoside showed practically no
absorption above 310 nm as noted from Fig. 1, it significantly
improved the survival ratio after irradiation with UVB as
10 shown in Table 1. It is also clear from Table 2 that the
chromanol glycoside manifested only the preventing effect
in the production of IL- α capable of allowing induction of
ascorbic acid and glutathione by virtue of the ultraviolet
light but that the TMG was confirmed to combine this effect
15 with the curing effect and prove effective in preventing and
curing the inflammatory disease on the skin.

Test for confirming improving effect relative to deposition
of ultraviolet light-induced pigment

Pigment-sedimented models were produced from Al-1 type
20 colored guinea pigs (female, 7-week old) divided into groups
of 6 heads by shaving their backs and performing the irradiation
of the skins of the backs with the ultraviolet light (light
source: xenon lamp, dose: 2 MED \times 1 minute) once daily up
to a total of three repetitions three to four days apart.
25 After the models had been left standing for 10 days from thence,

	ΔL value
TMG-applied group	2.00
Control group	0.05

It is clearly noted from Table 4 that the pigment sedimented by the ultraviolet light was lightened significantly by the application of chromanol glycoside and that the dermatological agent for external use of this invention possessed of the action of allaying the sedimentation of pigment by the ultraviolet light.

V79 or NB1RGB was adjusted with a culture medium till the cell density reached 5×10^4 pieces/ml. Then, it was sown in a unit volume of 100 μ l in the component wells of a 96-hole plate and cultured in an atmosphere of 5% CO₂ at 37°C for 72

hours. The culture medium used herein was the ordinary culture medium. Some of the wells effecting culture in the ordinary culture medium containing 100 μ M of chromanol glycoside formed a chromanol glycoside-added group and the remainders of the wells effecting culture in the ordinary culture medium formed a control group. The groups each consisted of 80 samples. After the elapse of 72 hours from thence, a neutral red reagent (0.015%) was added in a unit volume of 100 μ l to the wells and the resultant contents of the wells were cultured for 3 hours. After the 3 hours' culture, the culture medium was removed and a fixing solution (aqueous solution containing 0.5% of formaldehyde and 0.1% of calcium chloride) was added in a unit volume of 200 μ l to the wells. The resultant contents of the wells were left fixing for one minute and then the fixing solution was removed. Subsequently, an extraction solution (aqueous solution containing 50% of ethanol and 1% of acetic acid) was added in a unit volume of 100 μ l to the wells. The resultant contents of the wells were left standing for 20 minutes and measured for absorbency at 490 nm by the use of a micro-plate reader so as to allow computation of the number of cells. On the basis of the results of the measurement, the relative breeding ratios of the samples were determined, with the number of cells in the control groups taken as 100%. The results are shown in Table 3.

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		Breeding ratio (%)	
		V79	NB1RGB
Control group		100	100
Chromanol glycoside -added group	TMG	112	118
	TMGA	115	121
	TMFR	126	115
	TMMA	124	121

Test for acute toxicity

Production Example 1

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Production Example 2

An ointment was obtained by heating 2 g of TMG, 6 g of liquid paraffin, 2 g of bees wax, 3 g of a self-emulsion type monostearic acid glyceride, and 5 g of white soft paraffin till they were dissolved and dispersed.

Production Example 3

A cosmetic cream was obtained by thermally dispersing 2 g of TMG, 2 g of monostearic acid glyceride, 4 g of stearyl alcohol, 2 g of octyl dodecanol, and 5 g of monooleic acid polyoxyethylene sorbitan and subjecting the resultant dispersion together with a solution obtained by thermally dissolving 0.1 g of methyl paraoxybenzoate, 5 g of glycerin, and 60 g of purified water to emulsification by high-speed agitation, and cooling the produced mixture.

Production Example 4

A toilet lotion was obtained by mixing 2 g of TMG, 5 g of ethanol, 5 g of 1,3-butylene glycol, and 0.05 g of perfume in 100 g of purified water till dissolution.

Industrial Applicability

The dermatological agent for external use of this invention has as an active component thereof a chromanol glycoside which exhibits solubility in water and possesses an excellent activity to resist oxidation as described above. It is therefore capable of effectively eliminating active oxygen and free radicals generated by the ultraviolet light on the surface of the skin and in the tissue of the skin, repressing the dermatopathy, and allowing rapid amelioration of the morbidity.

The dermatological agent for external use of this invention is further capable of curbing the spread of the cutaneous inflammation by effectively repressing the production of cytokine induced by the ultraviolet light in

the local site of inflammation.

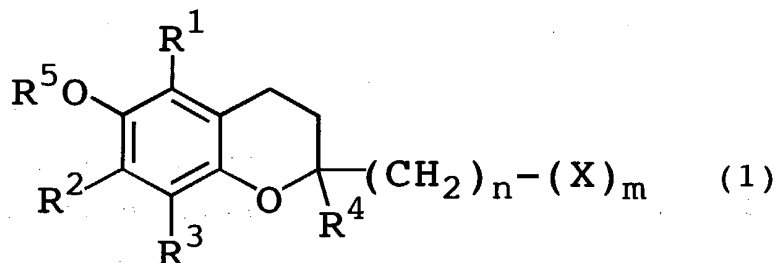
Further the dermatological agent for external use of this invention is capable of allowing unusually effective activation of the fibroblast which synthesizes such a matrix component as collagen in the skin, activating the metabolism of collagen and hyaluronic acid, enhancing the flexibility and elasticity of the cutaneous cells, promoting the phenomenon of turnover, repressing the sedimentation of pigment in the skin, and promoting the beautification of the skin in white.

Since the dermatological agent for external use of this invention has as an active component thereof a chromanol glycoside abounding in solubility in water, it can be formulated as an aqueous pharmaceutical preparation which contains the active component in a high concentration and enjoys high stability of preservation. Moreover, since this agent excels in the percutaneous absorbency, it is capable of being percutaneously administered as an external medicine to the affected region, effectively acting on the seat of disease even at a small application rate, preventing and curing the dermopathy, and warranting exceptionally safe use on account of the absence of a side effect.

The dermatological agent for external use of this invention, therefore, is exceptionally advantageous when it is used as an agent for preventing and curing disorders caused by the ultraviolet light, an agent for preventing and allaying the sedimentation of pigment in the skin, an agent for beautifying the skin in white, an agent for preventing the senescence of the skin, an agent for preventing and curing the dermatopathy as by activating cells, and a cosmetic preparation.

CLAIMS

1. A dermatological agent for external use containing a chromanol glycoside represented by the following general formula (1)



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(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, X represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0 - 6, and m represents an integer in the range of 1 - 6).

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2. A dermatological agent for external use according to claim 1, wherein said chromanol glycoside is 2-(α -D-glycopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-galactopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, 2-(β -D-fructopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol, and 2-(α -D-mannopyranosyl)methyl-2,5,7,8-tetramethyl chroman-6-ol.

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3. A dermatological agent for external use according to claim 1 or claim 2, which is an aqueous pharmaceutical preparation.

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4. A dermatological agent for external use according

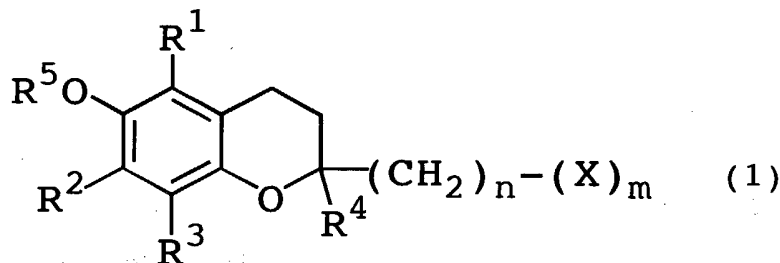
to any of claims 1 - 3, which is an agent for preventing and curing dermatopathy.

5. A dermatological agent for external use according to claim 4, which is an agent for preventing and curing the disorder caused by the ultraviolet light, an agent for preventing and allaying the sedimentation of pigment in the skin, an agent for beautifying the skin in white, an agent for preventing the senescence of the skin, or an agent for activating cells.

10 6. A dermatological agent for external use according
to any of claims 1 - 3, which is a cosmetic preparation.

ABSTRACT

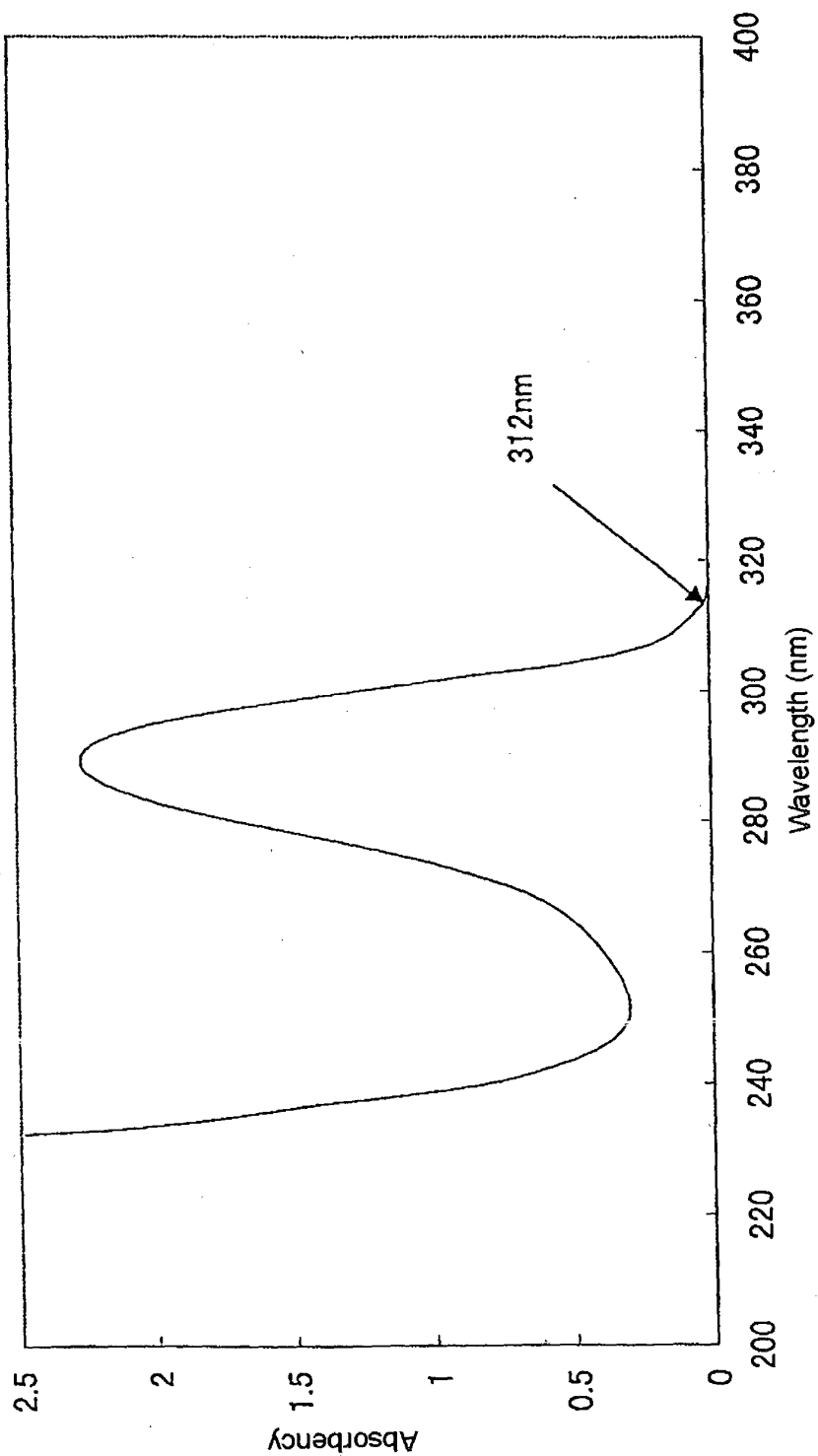
An dermatological agent for external use is disclosed which contains a chromanol glycoside represented by the following general formula (1)



5

(wherein R^1 , R^2 , R^3 , and R^4 , which may be the same or different, each represent a hydrogen atom or a lower alkyl group, R^5 represents a hydrogen atom, a lower alkyl group, or a lower acyl group, X represents a monosaccharic residue or an oligosaccharic residue optionally having the hydrogen atom of the hydroxyl group in the saccharic residue substituted with a lower alkyl group or a lower acyl group, n represents an integer in the range of 0 - 6, and m represents an integer in the range of 1 - 6). This is a novel dermatological agent for external use which excels in stability and percutaneous absorbency, manifests an effective action safely at a small application rate, and effectively prevents and cures the dermatopathy. It is very useful as an agent for preventing and curing the disorders caused by the ultraviolet light, an agent for preventing and allaying the sedimentation of pigment in the skin, an agent for beautifying the skin in white, an agent for preventing the senescence of the skin, an agent for activating cells, an agent for preventing and curing the dermatopathy, and a cosmetic preparation.

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Docket No.
4296-146 US

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
DERMATOLOGICAL AGENT FOR EXTERNAL USE

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on March 30, 2000 as United States Application No. or PCT International Application Number PCT/JP00/02034 and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

<u>11-093874</u>	<u>Japan</u>	<u>31/03/99</u>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	
<u>2000-022596</u>	<u>Japan</u>	<u>31/01/00</u>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	
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I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

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(Filing Date)

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(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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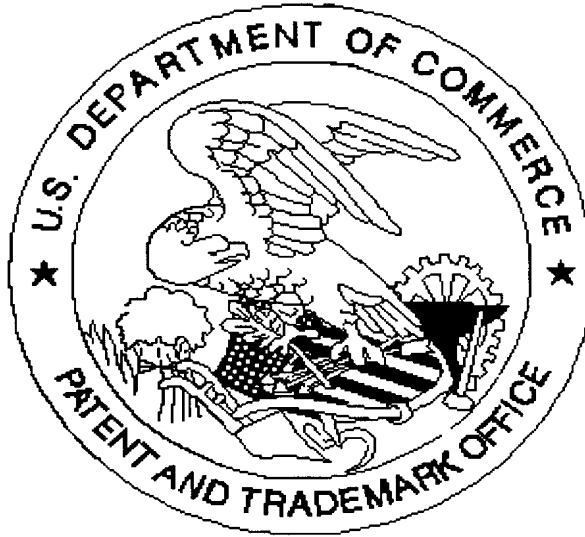
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